REMARKS

Claims 1-28 are pending in the subject application. By this response, the specification has been amended to reflect the current status of parent applications. Claims 1, 8, 9, 20, 25 and 26 have been amended and claims 19 and 24 have been canceled without prejudice. No new matter has been added by the aforementioned amendments. Support for the amendments is found throughout Applicant's specification and claims as originally filed. Entry of the amendments is respectfully requested. In view of the preceding amendments and remarks, reconsideration and withdrawal of the objections and rejections set forth in the April 21, 2004 Office Action is respectfully requested.

Claim Rejections - 35 USC § 112, second paragraph

Claims 1-2, 4-28 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for allegedly failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. This rejection is respectfully traversed.

Claims 1, 8, 9, 20, 25 and 26 have been amended and claims 19 and 24 have been canceled herein, thus rendering the instant rejections moot. Accordingly, reconsideration and withdrawal of the rejections is respectfully requested.

Claim Rejections – 35 USC § 103

Claims 1-2, 4-7, 9-17 and 19-28 stand rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Van der Zee et al., *Eur.J.Immunol.* 19:43-47 (1989) in combination with one or more other secondary and tertiary references (Lam et al., US Pat No. 5,510,240; Engehard, *Current Opin. Immunol.* 1994 6:13-23; Melief et al., US Pat No. 5,554,724). Applicant submits that Van der Zee is misapplied to the presently claimed invention, as detailed below, and thus, Applicant respectfully traverses these rejections.

1. Van der Zee does not teach the use of T cells, oligopeptides and antigen presenting means, each of which correspond to the same MHC-haplotype restriction.

Applicant respectfully asserts that, neither Van der Zee, nor any of the cited secondary references, teach or suggest any method to identify cytotoxic T cell epitopes wherein each of the assay components is correlated for MHC-haplotype status. By the present invention, (a) the

cytotoxic T cells all share the same MHC-haplotype restriction ("a population of cytotoxic T cells having the same MHC-haplotype restriction"), (b) the released molecules or peptides all come from a library based upon the MHC-haplotype restriction of those cytotoxic T cells ("contains a structural motif corresponding to an agretope of the MHC-haplotype to which said cytotoxic T cells are restricted"), and (c) the antigen presentation means is also based upon the MHC-haplotype of the cytotoxic T cells ("which antigen presentation means correspond to the MHC-haplotype to which the cytotoxic T cells are restricted"). The correlated cytotoxic T cells, library of molecules and the antigen presentation means permits complete testing of a less complex library with the goal of finding a range of active molecules, including but not limited to the native sequence.

Since "a population of cytotoxic T cells" of the instant invention have the "same MHC-haplotype," only one MHC-haplotype is defined at a time. Since each MHC-haplotype is defined by a different structure, a different peptide library will be used for each agretope of the MHC-haplotype. In this manner, each released oligopeptide will correspond to the same agretope of the MHC-haplotype (and to which the cytotoxic T cells are restricted).

This concept of using peptide libraries based upon MHC-haplotype status of the population of cytotoxic T cells to be tested is not found in Van der Zee (or, to Applicant's knowledge, in any other prior teaching). The present invention offers advantages of reducing complexity of the library, while at the same time preserving a potential of finding every reactive member of the library if desired.

This goal of seeking enumeration of multiple peptides capable of eliciting a cytotoxic response when complexed with a cytotoxic T cell and antigen presentation means is also not found in the prior art. According to the teachings of Van der Zee, it would not be useful to create degenerate libraries conserved only for MHC-haplotype because, according to Van der Zee, all native residues are seen as essential[1].

Moreover, since Van der Zee's assay relies upon syngeneic thymocytes as antigen presentation means (see, Van der Zee at Section 2.2), it would not be necessary for Van der Zee to correlate the MHC status of the cytotoxic T cells, the oligopeptides and the antigen

[1] See, Van der Zee et al. at page 44, col. 1 "By synthesis of variant peptides evidence was obtained that teach each residue of a seven amino acid long sequence is essential to T cell stimulation."; page 46, col. 1 "Of all nine possible variant peptides (9 amino acids long), full reactivity was only found with peptides having alanine substitutions of residues 187 and 188. All substitutions of residues 180 through 186 resulted in a complete loss of T cell stimulation activity."; page 46, col. 2 "Deletion peptides that lacked any amino acid in the 180 to 186 region were unable to stimulate both T cell clones. . . "; page 47, col. 1 "Apparently all residues in the relatively short 180-186 sequence are required . . ."

presentation means. By definition, a syngeneic system is from the same organism.[2] Thus, syngeneic thymocytes have a repertoire of all the necessary antigen presentation means for each T cell tested, and therefore, need not be matched for MHC-haplotype. Distinguishable from the aforementioned syngeneic system, is a scenario where one contacts a population of cytotoxic T cells with antigen presenting cells displaying peptides that are not correlated for MHC haplotype. In this scenario, cytotoxic T cells will not recognize the antigen presenting cell:peptide complexes and, thus, only random, fortuitous T cell activity will be detected.

Van der Zee created several limited libraries; all closely based upon the native sequence of the 65-kDa mycobacterial protein, and, in fact, only modified one residue at a time. Specifically, Van der Zee tested 28 overlapping peptides for mapping of the native epitope (see, Van der Zee at Table 2), and, for characterization of the necessary residues an additional nine variants containing a single alanine substitution per peptide (see Van der Zee at Table 3) together with several deletion peptides (see Van der Zee at page 46, col. 2). Van der Zee found that all residues of the minimal epitope and seven of the nine residues (of the full epitope) were essential for stimulation of the T cell clones and noted that each of these residues were required. (See, Van der Zee at page 47, first paragraph). Thus, if one were to make libraries based upon the teachings of Van der Zee, they would contain only one difference per peptide as compared with the native antigen. Van der Zee uses degenerately designed sequences to sequentially study the contribution of each native residue.

Van der Zee goes on to comment on work done by other researchers in the field, also using single amino acid substitutions, which found that modification of certain residues could result in higher activity. Taking Van der Zee's data in view of these other researchers, at page 47, Van der Zee still concludes that all residues of the native epitope sequence are required for T cell stimulation.[3]

In addition, prior to the present invention, it was not appreciated that one would be able to meaningfully detect cytotoxic T cell activation when more than one peptide species having the requisite MHC-haplotype was released and evaluated in a single ("competitive") assay along

[2] See, e.g., The American Heritage Dictionary of the English Language: Fourth Edition (2000) "syngeneic: genetically identical or closely related, so as to allow tissue transplant; immunologically compatible."; Merriam-Webster Dictionary (2000) "syngeneic: genetically identical esp. with respect to antigens or immunological reactions."

[3] "In other studies investigating single amino acid substitutions at all positions within a defined sequence critical for proliferation, the replacement of some residues resulted in equal or even higher stimulatory activity...The present study shows that the occurrence of such indifferent residues is not a general feature of T cell epitopes. Apparently, all residues in the relatively short 180-188 sequence are required for association either with the MHC molecules, or with the T cell receptor or with both." (Van der Zee at page 47, col. 1).

with other released peptide species. The instant inventor determined that it would be possible to detect activity elicited from individual species even in a pooled fraction of peptides. Thus, unlike Van der Zee wherein a separate assay is conducted for each peptide species detached from individual rods which are arrayed in a microtiter plate pattern[4], by the present invention, a quantity of peptide is released from each of the solid phase supports in the library of oligopeptides.[5] The array format taught by Van der Zee is spatially addressable thus allowing one to immediately know the amino acid sequence of each peptide (without purification or sequencing) based on its respective position or "address" in the array. Therefore, in contrast to the present invention, Van der Zee does not teach or suggest 'competitive evaluation'.

In view of such teachings, one skilled in the art would not be motivated to release quantities of peptides from a plurality of solid phase supports in a library (necessitating simultaneous testing of multiple peptide species), thereby losing one's ability to (i) determine individual stimulatory index values for each of the multiple peptide species simultaneously assayed; and (ii) immediately know the amino acid sequence of the peptide specie(s) generating the observed activity.

2. Van der Zee teaches away from the present invention.

Applicant notes that Van der Zee et al. teaches away from the presently claimed invention for a variety of reasons:

Firstly, none of Van der Zee's derivatives were significantly superior to the native epitope. Therefore, other than for mapping or characterization of a known T cell epitope, one skilled in the art would not be motivated to prepare or analyze derivative epitopes.

Review of the data presented by Van der Zee in Tables 2, 3 and 4 readily demonstrates that no derivatives showed significantly enhanced ability to stimulate T cell activity. Moreover, Van der Zee notes that the majority of residues was absolutely required for activity (See, footnote 3 *supra*). They cite work by other researchers finding that replacement of certain

[4] "Therefore, in the present study we developed a procedure for the detachment of the peptide from the solid phase using mild conditions after their simultaneous synthesis by the automated PEPSCAN method." (Van der Zee at sentence bridging pages 43-44). Van der Zee describes the PEPSCAN method: "With this easily automatizable procedure small amounts of several hundreds of peptides are simultaneously synthesized on activated polyethylene rods arrayed in a microtiter plate pattern." (Van der Zee at page 43, col. 2).

[5] Peptide libraries that are synthesized in array format, such as the library disclosed by Van der Zee et al., are spatially addressable. Distinct from peptide libraries that are 'mixtures', the amino acid sequence of each peptide (by virtue of its position in the array) is immediately known without purification or sequencing.

residues can result in equal or higher stimulatory activity; but, given Van der Zee's data, the authors conclude "indifferent residues is not a general feature of T cell epitopes." It seems clear that Van der Zee makes the case that the method is useful for mapping and characterization, but not more.

Secondly, all derivatives tested by Van der Zee were based upon the known native sequence. When different than the native, such derivative contained only one change per peptide. Therefore, if one skilled in the art sought to make and test derivative epitopes according to Van der Zee, the native epitope would be used as a template with only single residue changes.

There is no reason to expend the significant time and energy to create libraries of peptides sharing the same MHC-haplotype based upon Van der Zee. Van der Zee's teaching would not encourage others to expend time or resources in a broad search for non-native epitopes. It is clear that a disciple of Van der Zee would to look skeptically at the time and energy spent in creating a library of derivatized natural epitopes based upon any criteria other than based upon a known sequence.

As discussed above, if one has identified the natural epitope according to Van der Zee, there is no reason to look further. Contrary to the conventional teaching at the time the present invention was filed, however, the instant inventor is actively seeking a method to identify a wide range of "derivatized natural epitopes" (i.e., non-natural or altered ligands). The inventor has determined that non-native ligands offer improved immunological reactivity and hence, developed his method to identify each molecule or peptide specie that elicits the cytotoxic T cell response in a given library.

Notwithstanding the efforts to date to identify T cell epitopes, the inventor has recognized a clear need in the art for a rapid method to identify cytotoxic T cell epitopes In several cases, derivatized natural epitopes are more effective than the natural epitope itself, accordingly, there is a need to identify such derivatized natural epitopes.

3. Van der Zee does not teach detecting cytotoxic T cell activation by evaluating lysis of the antigen presentation means by the activated cytotoxic T cells.

The invention method is directed to the identification of cytotoxic T cell epitopes by evaluating activated cytotoxic T cells. As such, the method requires that the activity of the T cells, elicited upon the formation of a trimolecular complex of an antigen presentation means/a

single species of released oligopeptide/a cytotoxic T cell, is cytolytic activity, wherein the activated cytotoxic T cells lyse the antigen presentation means.

Applicant notes that the teachings of Van der Zee do not teach or suggest cytolytic T cell activity or lysis of antigen presentation means. Rather, Van der Zee relies upon the use of a T cell stimulatory (or proliferation) assay, as set forth in Section 2.2, in order to map and characterize the peptides tested.

Applicant does not challenge the usefulness of such stimulatory assays to measure T cell activity. In some embodiments, one may choose to use a stimulatory assay in addition to detecting the cytolytic activity. However, it is required that each peptide specie identified by the methods of this invention be capable of eliciting cytolytic activity when presented by an antigen presenting cell and complexed with a cytotoxic T cell. The Office can readily appreciate that T cell epitopes capable of eliciting lytic activity will have advantages when used in a therapeutic context as compared with T cell epitopes which merely stimulate the production of T cells.

4. The deficiencies of Van der Zee are not cured by the combination with Lam et al.

Applicant respectfully disagrees with the assertion (page 8, lines 4-7, April 21, 2004 Office Action) that it would have been obvious to one skilled in the art at the time the invention was made to use selectively cleavable linkers to attach the peptides to beads taught by Lam et al. in the method of Van der Zee et al. with the expectation of identifying T cell epitopes . . . and synthesizing variants of the epitope.

Contrary to the above assertion, one of ordinary skill in the art would not be motivated to combine the selectively cleavable linkers of Lam et al. in the method of Van der Zee et al.

First, Van der Zee et al. already discloses means for detaching (i.e., cleaving) peptide from the supports.[6]

Second, Van der Zee et al. admits that the quantity or amount of peptide cleaved from the supports: (i) are adequate (enabling analysis) for several experiments; and (ii) correspond to about 10% of the amount present on the supports before cleavage (i.e., leaving peptide on the support available for further analysis).[7]

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[6] See, Van der Zee et al., at page 43, abstract, lines 11-13; at page 43, col.2 "in the present study we developed a procedure for the detachment of the peptide from the solid phase under mild conditions"; at pages 44-45, section 2.1 and section 3.1; at pages 46-47, section 4.

[7] See, footnote 6, and Van der Zee et al. at page 45, col.2, lines 6-11; at page 47, col.2, lines 1-4.

Accordingly, if one of ordinary skill in the art could achieve cleavage of a quantity of peptide from the support adequate for analysis while also retaining a quantity of that peptide on the support, available for additional analysis, as disclosed in Van der Zee et al., the logic supporting the asserted "motivation" (page 8, Office Action) clearly fails. Therefore, the asserted "advantages" of using selectively cleavable linkers (page 8, lines 7-12, Office Action)

> such that only a fraction of peptides are cleaved from the beads to identify T cell epitopes taught by Van der Zee et al. and still have peptides attached to the beads which would be useful in structure analysis methods . . .

are moot, because those alleged "advantages" are clearly taught in the method of the Van der Zee et al. reference.

Furthermore, if one of ordinary skill in the art did combine the selectively cleavable linkers of Lam et al. in the method of Van der Zee et al., such combination would neither teach nor suggest the claimed invention. As discussed above, the "combination" would not place the artisan in a better position than the artisan would have if employing only the method of the primary reference.

Given the clear failure of Van der Zee et al.,(taken alone or when combined with Lam et al.), as discussed hereinabove, it unmistakably follows that the deficiencies of Van der Zee are not cured by the combination with Lam et al. and further combined with Engelhard. Similarly, the deficiencies of the primary reference are not cured by the combination with Melief et al.

In view of the above remarks, reconsideration and withdrawal of the rejections under 35 U.S.C. § 103 is respectfully requested.

CONCLUSION

No fee is deemed necessary in connection with the filing of this Communication. However, if any fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 07-1074.

Respectfully submitted,

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